

Age and early graft function relate with risk-benefit ratio of allogenic islet transplantation under Anti-thymocyte globulin – Mycophenolate mofetil – Tacrolimus immune suppression

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Clinical Trial registration: ClinicalTrials.gov NCT00623610 and NCT00798785

Contribution statement:

D.L., B.K. and P.G. contributed to the study design, literature search, data collection, data analysis and interpretation and writing of the manuscript. R.H., U.V.d.V., and Z.L. researched data and reviewed the manuscript. D.J.T.T., G.M. contributed to data research and reviewing of the manuscript. B.L., C.D.B., L.C. contributed to the patient recruitment, data research, and reviewing of the manuscript. C.M. researched the data and reviewed the manuscript. D.P. contributed to the discussion and editing of the manuscript. P.G. and B.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosures:

The authors declare no conflicts of interest.

Funding:

This study was supported by the Juvenile Diabetes Research Foundation (JDRF grants 4-2001-434, 4-2005-1327), the Research Foundation Flanders (FWO Vlaanderen projects WO 040.04. G.0311.07-10. G.0800.09N, senior clinical research fellowship for BK), the W. Gepts Fund of University Hospitals Brussels (project 71046) and the Vrije Universiteit Brussel (project OZR1915). P.G. is funded by the Clinical Research Foundation of the University Hospitals Leuven, Katholieke Universiteit Leuven. B.K. is senior clinical investigator of the Research Foundation Flanders (Fonds Wetenschappelijk Onderzoek-Vlaanderen).

Abbreviations

AE, Adverse events

ATG, Anti-thymocyte globulin

CMV, Cytomegalovirus

CVfg, Coefficient of variation of fasting glycemia

IQR, Interquartile range

IS, Immune suppression

MMF, Mycophenolate mofetil

Pre-Tx, Pretransplantation

PT, Posttransplantation

SAE, Serious adverse events

Tac, Tacrolimus

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Abstract

Background: Induction therapy with a T cell depleting agent followed by mycophenolate mofetil (MMF) and tacrolimus (Tac) is presently the most frequently used immune suppression (IS) regimen in islet transplantation. This study assesses its safety and tolerability in nonuremic type 1 diabetic recipients.

Methods: Fifty-one patients (ages between 29-63 years) with high glycemic variability and problematic hypoglycemia received intraportal islet grafts under anti-thymocyte globulin (ATG)-MMF-Tac protocol. They were followed over 48 months for function of the implant and adverse events.

Results: Severe hypoglycemia and diabetic ketoacidosis were absent in patients with functioning graft. Immune suppressive therapy was maintained for 48 months in 29 recipients with sustained function (group A) while in 16 patients stopped earlier due to graft failure (group B) and in 6 for other reasons. Group A was significantly older at the time of implantation and achieved higher graft function at posttransplant (PT) month 6 under similar dose of IS. Prevalence of IS-related side effects was similar in group A and B, occurring predominantly during the first year PT. IS-related serious adverse events (SAE) were reported in 47% of patients, with 4 presenting with CMV infection and 4 (age 42 to 59 years) diagnosed with cancer. Except in 1 patient with cancer, all SAE resolved after appropriate treatment.

Conclusions: These risk/benefit data serve as a basis for clinical decision-making before entering an intraportal islet transplantation protocol. A longer benefit is observed in recipients of higher age (≥ 40 yrs), but it is not associated with more side effects and SAE.

Introduction

For type 1 diabetic patients with problematic hypoglycemia, islet transplantation represents a possibility of therapy (1-3). However, the risk benefit ratio needs to be determined. This is not easy in view of the heterogeneity of the recipients and in the transplant protocols. Single center protocols usually have standardized follow-up of graft function, metabolic control and safety outcome measurements (2,4-7). Because of the limited number of patients per center, analysis is often conducted in a combination of preuremic and kidney transplant recipients and of patients under different immunosuppressive regimens (8). In Belgium, a collaborative Islet Transplant Network was formed, with islets being isolated in the central isolation facilities of the Diabetes Research Center of the Vrije Universiteit Brussel (VUB) and transplantations being performed by a collaborative team in 2 Belgian academic centers (6). The present study examines risk-benefit ratio of well-characterized human islet cell grafts in nonuremic type 1 diabetic patients receiving immune suppressive therapy that consists of anti-thymocyte globulin (ATG) as induction and mycophenolate mofetil (MMF) plus tacrolimus (Tac) as maintenance. This combination of T-cell depletion and MMF-Tac is currently the most frequently used regimen in islet transplantation (9) and has been used in our program for more than 15 years (10).

Materials and Methods

Graft Recipients

Data were collected from a total of 51 islet beta cell recipients with type 1 diabetes mellitus complicated by hypoglycemia unawareness, transplanted between December 2001 to March 2011. Baseline patient characteristics are shown in Table 1. The recipients were included according to the following criteria: 1. Nonsmokers ages between 18 to 65 years; 2. C-peptide negativity defined as plasma C-peptide < 0.09 ng/ml at glycaemia of 120 – 200 mg/dL; 3. Signs of chronic diabetic complications (microalbuminuria despite optimal dose of angiotensin-converting enzyme inhibitor, retinopathy, or hypoglycemic unawareness); 4. Large within-person between-day variation in fasting self-monitored plasma glucose (defined by coefficient

of variation fasting glycaemia [CVfg] $\geq 25\%$ and HbA1c $\geq 7\%$ or 53 mmol/mol despite 4 daily subcutaneous insulin injections or pump therapy. Female patients of childbearing potential were excluded. Ethics Committee of the Belgian Diabetes Registry (BDR) and the participating university hospitals approved the protocol. This study was performed according to the Declaration of Helsinki and institution review board approval was obtained (institutional review board protocol BK/3 and BK/136; clinicaltrials.gov NCT00623610 and NCT00798785). A written informed consent was obtained from every patient.

Preparation of islet beta cell grafts and transplantation

Isolation and preparation of islet beta cell allografts was as described previously (6). The technique to access the portal vein depended on the expertise of the site where patients were transplanted, either by laparoscopy (University Hospital Brussels (11)) or by percutaneous fluoroscopic-guided trans-hepatic injection (University Hospital Leuven (12)). In total 91 grafts were transplanted of which 41 grafts (n=22 patients) were transplanted by laparoscopic and 50 grafts (n=29 patients) by percutaneous cannulation of the portal vein. Twelve patients received 1 implant, 38 patients 2 implants within 3 months (IQR 2.4-3.5 months) after the first implantation and 1 patient received 3 implants (at 2.5 and 5 months posttransplantation). Based on a possible beneficial effect of intravenous heparin infusion peritransplant as reported by Koh et al (13), heparin was administered in 3 patients undergoing laparoscopic implantation at 70 U/kg using continuous infusion of heparin containing saline (10U/ml) at 500 U/h (50ml/h) up to 24 hours post-transplantation (PT). After intra-abdominal bleeding in 2 of the 3 cases, heparin administration was removed from the protocol. Doppler ultrasonography of the portal vein and liver was performed within 24 hours PT to rule out bleeding and thrombosis.

Immune suppression and monitoring

The immune suppression regimen consisted of induction therapy with anti-thymocyte globulin (ATG-Fresenius®, Fresenius, HemoCare, Redmond, WA) and maintenance therapy with mycophenolate mofetil (MMF, Cellcept®, Roche, Basel, Switzerland, n=46) or mycophenolic acid (Myfortic®, Novartis, Basel,

Switzerland, n=5) and tacrolimus (Prograf(t)®, Astellas Pharma Europe, Staines, UK). First dose of ATG was given at day 4 (n = 21) or day 1 (n = 30) before the first implantation (9 mg/kg) and continued at 3 mg/kg for 6 days if T-lymphocyte count was above 50 /mm³. No additional ATG was administered at the second or third implantation. MMF (2g/day) or mycophenolic acid (1440 g/day) was started together with the first ATG injection and maintained at this dose, unless clinically necessary. All 5 patients who were started with mycophenolic acid were switched over to MMF within the first year after transplantation, according to availability. Patients received Tac from day 2 (n=33) or day 5 (n=18) at a dose to achieve serum trough levels of 8-10 ng/ml first 24 months and 6-8 ng/ml thereafter. Two hours before the first ATG administration and before each beta cell graft, 500 mg methylprednisolone was given intravenously.

Anti-infectious prophylaxis consisted of valganciclovir (Valcyte® 900 mg qd, Roche, Basel, Switzerland) and sulfamethoxazole/trimethoprim (Bactrim Forte® 800mg/160mg qd, Roche, Basel, Switzerland) during 100 days after each implantation.

Assessment of side effects and adverse events

Outpatient follow up was performed weekly until PT week 6, and every 2 weeks between PT week 6 and 12 and monthly thereafter. At each visit, a questionnaire was completed with a listing of possible adverse events and side effects, followed by a physical examination. Blood work was done for hematology, kidney and liver function, metabolic parameters, tacrolimus trough levels (Tacrolimus II, IMx Abbott; Abbott Laboratories, Wiesbaden, Germany) and cytomegalovirus polymerase chain reaction (Amplicor CMV-test; Roche Diagnostics). Adverse events (AE) were assessed and recorded following the Common Terminology Criteria for Adverse Events v3.0 (CTCAE), as recorded in the Clinical Islet Consortium (14), with the grading scale of 1, mild; 2, moderate; 3, severe; 4, life-threatening; and 5, death. Serious adverse events (SAE) were defined as fatal, life threatening, causing disability, causing or prolonging hospitalization, occurrence of malignancy or congenital anomaly, and requiring intervention to prevent permanent impairment or damage (15). The relationship between each adverse events and transplant protocol was

categorized by causality; definitely related, probably related, possibly related, probably not related, not related, and unknown. Events reported from grade 2 to 5 were included in our analysis.

Assessment of metabolic control and acute diabetic complications

Plasma C-peptide, glycaemia, and HbA1c concentrations were measured in the central laboratory of the Belgian Diabetes Registry (6). All patients performed 4- to 7-point glycaemia profiles of home blood glucose monitoring. Coefficient of variation of fasting glycaemia (CVfg) was calculated using the glycaemia measured from home blood glucose monitoring defined as the ratio of the standard deviation (δ) to the mean (μ): $CVfg = \delta / \mu$. Insulin dose-adjusted A1c (%) was calculated using $HbA1c (\%) + [4 \times \text{insulin dose (U/kg/day)}](16)$.

Graft failure was defined as consecutive random plasma C-peptide <0.2 ng/mL measured at glycaemia of 120-220 mg/dL. Hypoglycemic events were defined as blood glucose levels <70 mg/dL and were assessed by records of home blood glucose self-monitoring. Severe hypoglycemia was defined as an event requiring assistance of a third party to actively administer carbohydrate, glucagon, or other resuscitative actions that may be associated with sufficient neuroglycopenia to induce seizure or coma.

Diabetic ketoacidosis was defined as hyperglycemia (blood glucose >200 mg/dL) with metabolic acidosis (venous pH <7.3 or plasma bicarbonate <15 mmol/L) and the presence of ketones in blood or urine. This was documented with the use of patient reports and hospitalization records.

Implant function and metabolic control data were compared between 2 patient groups, those who remained under IS until 48 months (Group A) and those who stopped IS during the follow-up due to graft failure (Group B).

Statistical methods

Data collected were for intention-to-treat analysis. All values are expressed as median and interquartile range (IQR) or mean \pm standard deviation when indicated. For the comparison of the baseline patient characteristics, Pearson's Chi-squared test was used for categorical data and Kruskal-Wallis for continuous data in 3 different patient groups. To assess the difference between different time points during the follow up, Fisher's exact test was used for categorical data and Mann-Whitney U test was used for continuous data. All analysis was performed using SPSS (version 22.0) and the graphics were computed by using Graph Pad Prism (version 5.0). All reported p -values are 2-sided and $p < 0.05$ was considered statistically significant.

Results

Baseline patient characteristics of patients with continued and discontinued immune suppressive therapy

Of the 51 patients, 29 continued IS for 48 months (Figure 1). The treatment was stopped earlier in 22 patients (median duration 18 months (IQR 13-34)), in 16 because of a failing graft and in 6 for another reason in presence of a functioning graft. The latter group consisted of 2 patients diagnosed with cancer (malignant melanoma at month 13 PT; gastric adenocarcinoma with hepatic metastasis at month 27 PT), 1 suffering from therapy-resistant cytomegalovirus infection between month 6 and 13 PT, 1 who died at age 61 as a consequence of acute cerebral hemorrhage at month 4 PT and 2 who were disappointed that insulin-independence was not achieved (month 7 and 10 PT).

All 51 patients exhibited high pretransplant glycemic variability above 25% (Table 1). The majority had microvascular complications, with clinical macroangiopathy in 20 patients.

There were no differences in gender, body weight or BMI in the recipients who continued or discontinued IS. Patients with graft failure were significantly younger ($p=0.001$) and exhibited worse glycemic control at time of transplantation, shown as corrected insulin dose (higher insulin dose-adjusted A1c (16)) ($p=0.01$).

Serious adverse events

Of the 51 patients, 24 (47%) experienced SAE (Table 2). Three cardiovascular SAE were considered not to be study related.

SAE related to the implant procedure were uncommon; they occurred in 3 out of 91 implant procedures (3%): 1 percutaneous implant caused severe thoracic pain associated with pleuro-peritoneal irritation at the site of puncture (Table 2). No significant hemorrhage (defined as a drop in hemoglobin of more than 2 g/dl, or the need for transfusion or surgery) was seen after percutaneous islet transplantation. Two laparoscopic procedures were complicated by intra-abdominal bleeding when heparin injection was administered (2/3). Both cases received a blood transfusion without need for re-intervention. No portal vein thrombosis or puncture of the gallbladder occurred in either of the 2 approaches.

Thirty-one cases of IS-related SAE were reported in 19 patients. Four patients presented 2 or more SAE. Two patients exhibited signs of Tac-related toxicity: 1 with encephalopathy and 1 with nephrotoxicity confirmed by renal biopsy, both resolving after dose reduction. The most frequently reported SAE caused by infection was posttransplantation CMV infection, observed in 5 patients (10%) and successfully treated with antiviral medication and/or reduction of MMF. Four out of these 5 patients were CMV antibody negative before transplantation. The other CMV positive patient had reactivation of CMV at PT month 4. Four out of 5 patients developed CMV infection after prophylaxis was stopped. One recipient with de novo CMV infection needed an additional course of oral valganciclovir because of persisting CMV PCR titers after IV ganciclovir. Gastro-intestinal infections, possibly related to the intake of IS, were the most common cause of hospitalization, resolving with either antibiotics or supportive therapy.

There were 4 cancers reported; 1 metastasized gastric adenocarcinoma at age 56 with a fatal outcome at PT month 45, 1 acral lentiginous malignant melanoma at age 43 with complete remission after excision and cessation of MMF-Tac; 1 invasive prostate carcinoma at age 61 with complete resolution after total prostatectomy; and 1 invasive recto-sigmoid adenocarcinoma at age 47 with remission after laparoscopic

sigmoidectomy and cessation of MMF. Basocellular or squamous cell skin cancers or other nonskin cancers were not observed.

Side effects are most prominent during the first 12 months PT

During the first 12 months when the immune suppression dose was the highest, half of the patients experienced side effects but significantly decreased to 25% thereafter (Table 3).

The most prominent symptoms observed the first year were gastrointestinal symptoms, mostly pyrosis, nausea and vomiting, which significantly decreased from the second year after transplantation (Table 4). Neurological symptoms, mostly memory impairment and headache were observed in 22% of the patients the first year, but significantly decreased at the second year and remained present in around 5% of the patients. Respiratory, musculo-skeletal symptoms, as well as asthenia and skin symptoms were prominent during the first year, but also showed a decreasing trend from the second year until 48 months.

Laboratory abnormalities are most common during the first 6 months

Abnormal liver function tests were observed during the first month PT, with increases in aspartate transaminase (AST > 2.5 times upper limit of normal) in 27% of all patients from median day 6, quickly resolving at day 9 and alanine aminotransferase (ALT > 2.5 times upper limit of normal) in 43% from day 6, resolving at day 12. These abnormalities were not seen after the second islet infusion ($p < 0.001$ versus after first implant).

All patients developed a decrease in blood cell counts (Table 3), consisting of lymphocytopenia (100%), anemia (67%) and thrombocytopenia (8%) in the first weeks after first implantation. Blood cell counts normalized thereafter, except for lymphocytopenia, which remained present in a third of the recipients under immune suppression until month 48 PT (Figure 2). Twenty-five percent of the patients received transfusion

for anemia, but no erythropoietin treatment was required. One patient required therapy with granulocyte colony-stimulating factor (G-CSF). No platelet transfusions were given.

Implant function with metabolic control is sustained in 29 graft recipients

Maximal basal C-peptide levels were reached at 3-6 months PT in both the group who continued IS for 48 month (A; n=29) and those who stopped IS (B; n=16) during follow-up due to graft failure (Figure 3). In this period, C-peptide levels were twice as high in Group A than B, although after the first implantation (before month 3), a similar degree of beta cell implant function was measured in both groups. The insulin dose per kilogram in group A was reduced to half the dosage at this time, reaching nearly a third of the dose per kg of those in group B at 12 months ($p=0.002$) (Table 5). After month 12 PT, median C-peptide levels decreased at a similar pace in both groups.

A striking inverse relationship was observed between implant function and both glycemic variability and insulin dose-adjusted A_{1c} levels. None of the patient experienced ketoacidosis or severe hypoglycemic events during the entire duration of the study while this was the case for 6 (group A) and 20 (group B) patients in the year preceding the transplantation.

Tac trough levels as well as MMF dose were decreased with time, as defined in our protocol and there was no significant difference between group A and B observed (Table 5).

Discussion

This study shows that clinical benefits of islet transplantation outweigh the adverse events of chronic immune suppression in a large group of nonuremic type 1 diabetic patients. Patients with a functioning graft experience less glycemic variability with absence of severe hypoglycemia or episodes of ketoacidosis, without exposing them to unacceptable risks of chronic immune suppression. This positive risk-benefit ratio

is in line with the results of other groups (2,4,8,17-23) which studied more heterogeneous groups of islet-alone and islet-after-kidney transplantation recipients and/or using different immune suppression regimens. Our patient group received cultured islet grafts (6) that were standardized in terms of beta cell number, purity and time frame of a second transplant. In order to achieve these objectives, islet beta cells from more than 1 donor were combined in each implant. Immunosuppression consisted of ATG induction and MMF-Tac maintenance therapy, which is currently the most frequently used worldwide (9).

Out of the 51 islet recipients, more than half of the patients experienced at least 1 side effect during the 48-month follow-up. Both implantation procedures (laparoscopy(11) and transcutaneous(12)) were rarely associated with acute complications so that a negative impact on long-term islet function (24) was not seen in our cohort. Adverse events related to immune suppression were reported in 57% of patients. They occurred predominantly during the first year after transplantation, when the patients were treated with higher doses of MMF-Tac, together with the cumulative effect of ATG induction therapy and concomitant prophylactic medication. As observed in other transplant protocols (25-29), gastro-intestinal and neurological symptoms, well-recognized side effects of respectively MMF and/or Tac, were the most frequent in this study. MMF-related gastrointestinal symptoms rarely needed hospitalization and resolved after short-term supportive therapy. The majority of these AE resolved after dose reduction of immune therapy and side effects of grade 2 or more were observed in only a quarter of patients after the first year. In line with other reports (30-32), the majority of patients in the present study found the side effects less debilitating when compared to severe hypoglycemic episodes and unstable metabolic control and were satisfied with the transplantation.

In all patients, depletion of blood cells was observed in the first month after the first implant, caused by ATG induction, start of maintenance immune suppression and the administration of valganciclovir and trimethoprim-sulfamethoxazole. Frequent blood sampling in the peritransplant period may have aggravated anemia. Most patients presented with neutropenia during the first month, but this was not associated with

serious infections or high numbers of opportunistic infections. All patients had prolonged lymphocytopenia. The use of lymphocyte-depleting therapies is a well-recognized risk factor for cytomegalovirus infection (33-35), explaining the comparable CMV disease incidence in our cohort as in the study of Gala-Lopez et al (35) (9% vs 9.5%). It is also in line with lower CMV disease incidence observed in other islet transplant cohorts not using T-cell depletion (4,36). An additional important risk factor for CMV disease is the use of sero-positive organ donors in sero-negative recipients (37-39), which was the case in 4 out of 5 patients in our study. Except for 1 patient, CMV disease occurred more than 100 days after the transplantation, when prophylactic valganciclovir was stopped according to our protocol. Extending the duration of prophylactic therapy might prevent additional cases as shown after kidney transplantation (40).

Cancer is a well-established risk of chronic immune suppression (41). None of the patients in our cohort developed lymphoma or related diseases. Four patients with a well-functioning islet graft were diagnosed with cancer, 3 of them after year 2 PT. In 3 patients, diagnosis was made at an early stage, with immediate reduction (prostate cancer, colon cancer) or cessation (melanoma) of IS and follow-up has been so far uneventful. One patient died from liver metastasized gastric carcinoma. Although we cannot rule out the contribution of IS in the occurrence of this late stage gastric cancer, it is probable that an underlying pathology was already present before transplantation. This is probably also the case for the 3 SAE due to macrovascular events since they occurred in the early stages after transplantation (1, 4 and 18 months PT) in a patient population with already a high incidence of pre-existing macrovascular disease. Nevertheless, our findings emphasize the need for frequent monitoring of islet transplant recipients.

Another well-known risk of calcineurin-inhibitor based immune suppression is deterioration of native kidney function in recipients of a nonrenal organ (42). In a previous study, we already showed that the use of Tac caused an initial 20% reduction in eGFR, which was reversible following its discontinuation (43). Moreover, the risk-benefit ratio of IS regimen using sirolimus with lower levels of Tac is not better than IS regimens using MMF with higher levels of Tac (44). Our previous study using sirolimus-Tac or sirolimus

alone showed in patients with preexisting microalbuminuria, a worsening of albuminuria that was caused by sirolimus (45), supporting further use of a MMF-Tac based regimen in this patient population (43).

The most relevant clinical benefit for the patients was the absence of severe hypoglycemia posttransplantation. All patients also had significantly better (insulin dose-adjusted) HbA1c and a significant reduction in glycemic variability, which are well-known risk factors both for long-term complications and hypoglycemia (46-49). While C-peptide levels were comparable to patients with optimal graft function in other studies (2,7,9), maintenance of insulin independence was similar (2) or lower in our cohort. While this can be a sign of more rapid decline in beta-cell mass, our approach has always been to rapidly restart insulin if glucose values become abnormal pre- or postprandial, leading to early reintroduction of insulin.

The patient group where immune suppression was stopped because of loss of graft function had similar initial graft function (ie 0-3 months) as the group that maintained graft function during 48 months. The last group exhibited a 2-fold rise in graft function during the 3-6 months PT while no increase in graft function was measured in the patient group that failed within the first 4 years. This is in line with the publication by Vantghem et al (7), emphasizing the importance of optimal graft function 1 month after the last implant in maintaining long-term graft function with the Edmonton protocol.

The patient group who became C-peptide negative during follow-up was significantly younger. There are no surrogate markers that allow us to examine the possible reasons for this difference in outcome. MMF and Tac levels were not different with the first year PT but the patients with less favorable outcome were significantly younger and had a significantly worse baseline glucose control. Because HbA1c was not different at 0-3 months PT, we hypothesize that immune-related factors rather than metabolic factors are responsible for the worse outcome. It is well established in other organ transplantation settings that older age is associated with improved transplant survival and lower rates of rejection (50). Another reason for worse graft function in younger patients might be less compliance with the immunosuppressive regimen

(51,52). Future islet transplant protocols can explore the risk-benefit ratio of more potent immune suppressive regimen, especially in younger subjects.

In our cohort under immune suppression during 48 months, a decline in implant function started already after the first year. It is unknown whether this decline is due to a reduction in Tac and/or MMF and/ or a limited graft beta cell mass that is vulnerable to metabolic stress. Studies are ongoing that aim to reduce the important loss of functional beta cells immediately after intraportal transplantation (53-56) (ClinicalTrials.gov Identifier:NCT00789308). A phase 1 study with embryonic stem cell derived endocrine cells (57) has also been started (ClinicalTrials.gov identifier: NCT02239354); this new cell source may overcome the barrier of the limited amount of cells that is available for transplantation when deceased donors are used.

In conclusion, islet transplantation using ATG in combination with MMF-Tac is safe to use long-term, with side effects mostly limited to the first year with the maintenance of metabolic benefit in those with good initial graft function.

Acknowledgments

The authors thank Monique Robyn, Brigitta Swennen, Katrien Rouffe, and Nadine Pardon (University Hospitals Leuven); Soniya Thomas, Valerie Van Damme (Diabetes kliniek UZ Brussel) for their work in the clinical follow-up of the patients. The authors thank Cindy Tettelin and Veerle Kemels for data entry and co-ordination of the logistics. The authors also thank the Euro- transplant International Foundation and its transplant surgeons and coordinators for organ procurement. Finally, the authors thank the staff of the Beta cell Bank, the Belgian Diabetes Registry, the Diabetes Research Center of the Vrije Universiteit Brussel, and the Clinical Biology Department of UZ Brussel.

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Figure Legends

Figure 1: Flow chart of number of patients on immune suppression therapy following beta cell transplantation.

Figure 2: Depletion of blood cells in nonuremic type 1 diabetic recipients of long-term cultured islet beta cell allograft under immune suppression. Pre-Tx: pre-transplantation, IS: Immune suppression

Figure 3: Implant function and metabolic control in diabetic recipients of long-term cultured islet beta cell allograft. Data are shown as mean \pm SEM. Random C-peptide measured when glycaemia between 120-220 mg/dl. Group A: Under immune suppression for 48 months, Group B: immune suppression stopped before 48 months due to graft failure. IS: immune suppression, CVfg: coefficient of variation of fasting glycemia, IDAA1c: Insulin dose-adjusted hemoglobin A1c. Statistically significant compared with Group B * $p < 0.05$, $^{\$}p < 0.005$.

Figure 1

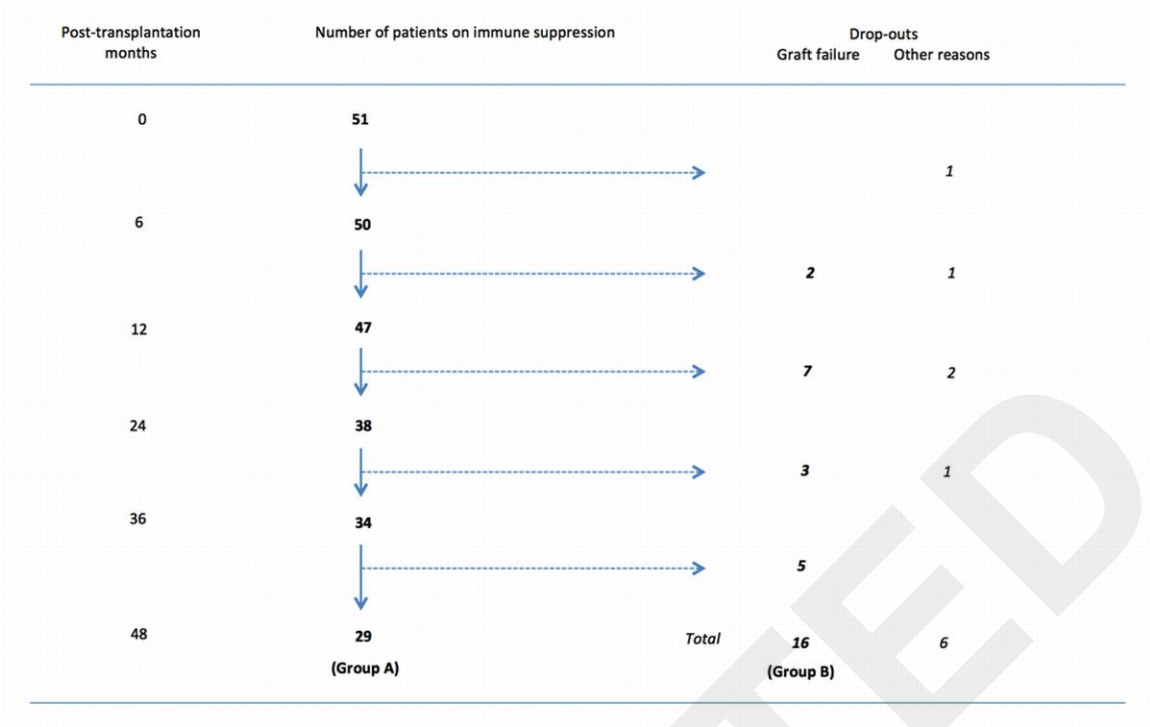


Figure 2

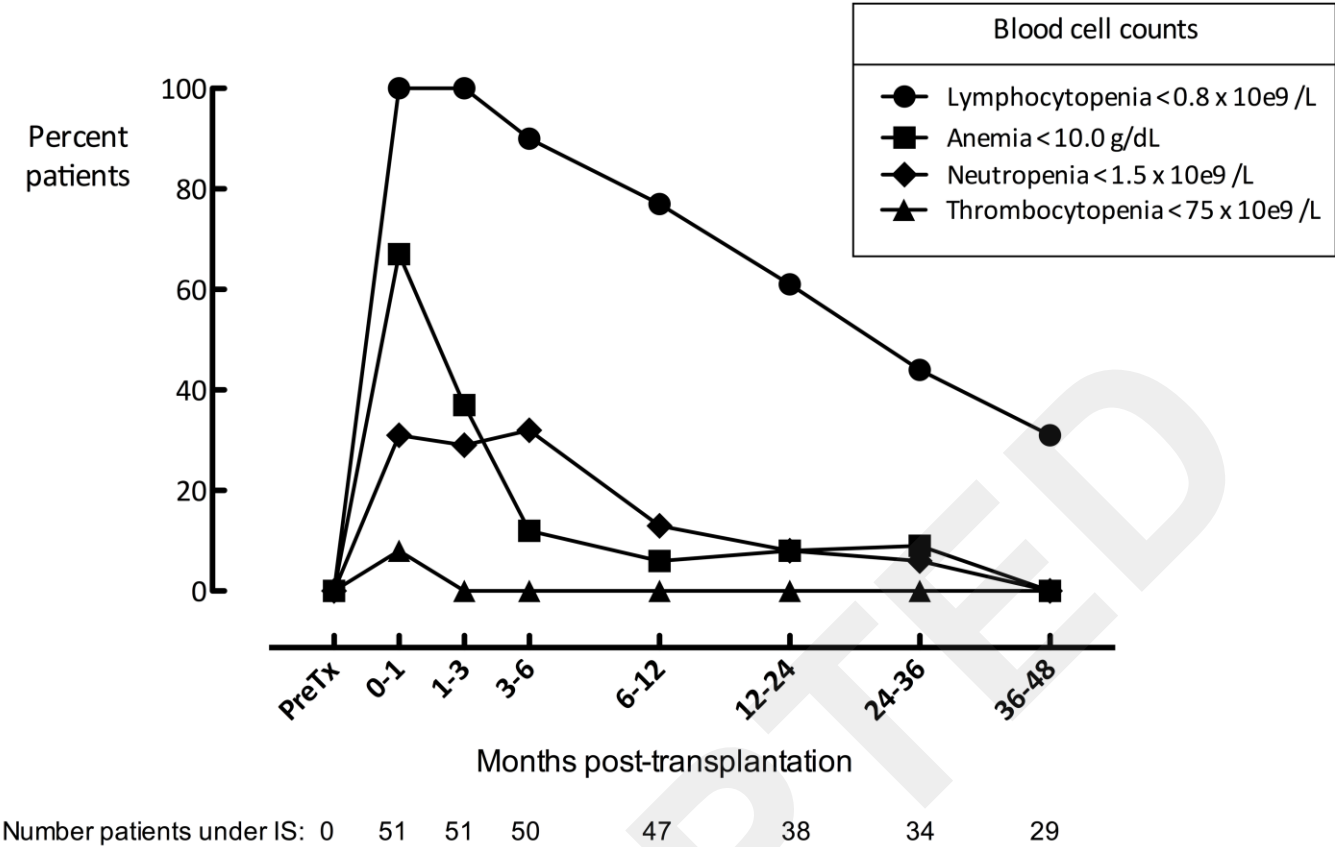


Figure 3

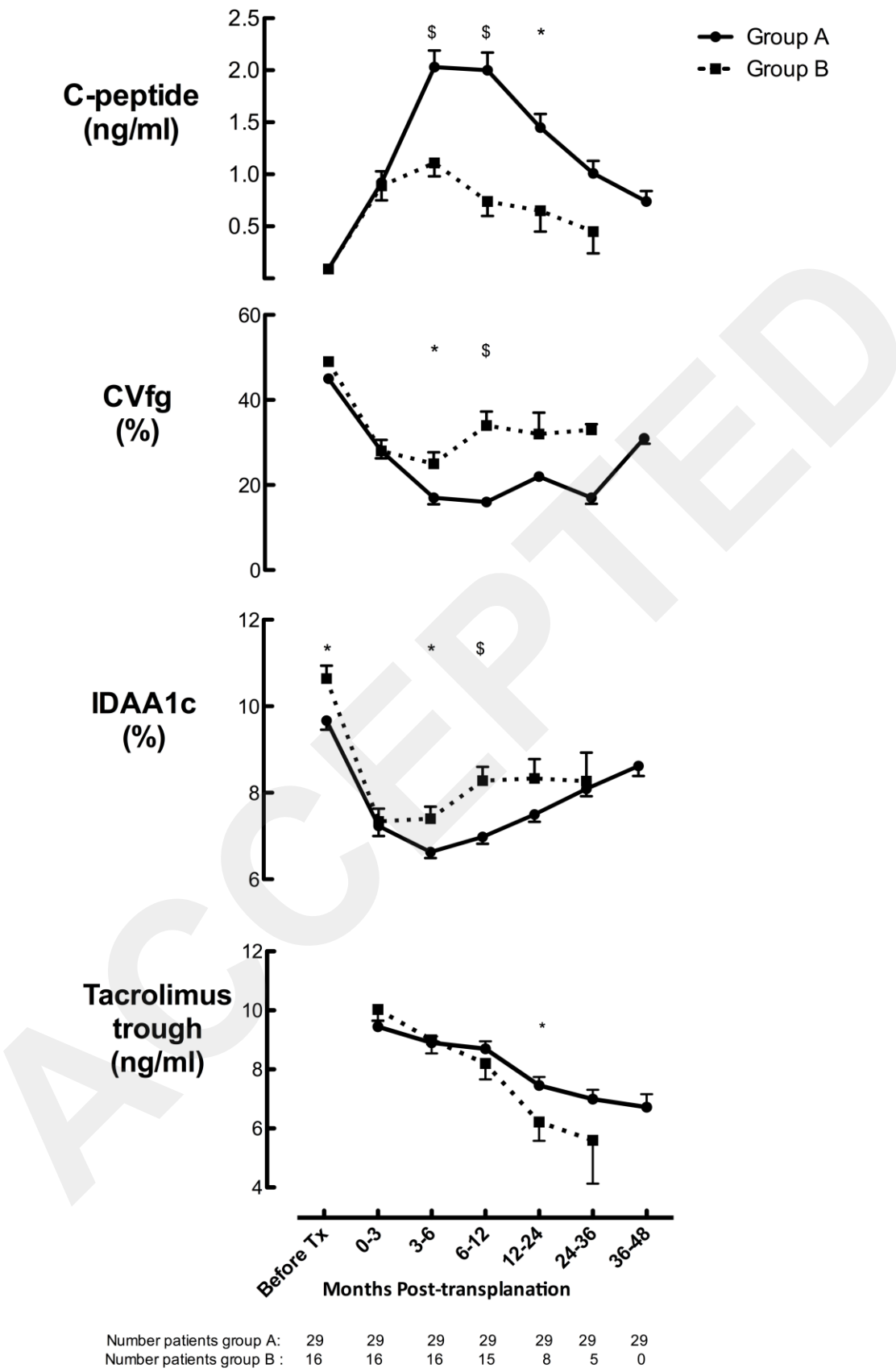


Table 1: Patient and Graft characteristics

Immune Therapy	48 months	Stop during 48 months	
		Graft Failure	Other reasons
n	29	16	6
General			
Male/Female	17/12	9/7	3/3
Body weight (kg)	66 (63-70)	70 (67-77)	69 (63-76)
BMI (kg/m ²)	23 (22-25)	24 (22-26)	25 (23-28)
Age at first islet transplantation (years)	46 (40-54)	37 (32-43) ^{\$}	50 (44-55)
Diabetes			
Age at clinical onset (years)	15 (12-26)	11 (8-22)	30 (14-37)
Duration of disease (years)	28 (24-38)	23 (20-29)	17 (12-42)
Positivity for ICA/GADA/IA2A/I(A)A	5/13/7/26	3/3/6/14	0/2/2/6
≥ 1 Auto-antibody positivity (%)	97	94	100
Auto-antibody negative (%)	3	6	0
HbA1c (%)	7.5 (6.9-8.2)	8.1 (7.4-8.8)	7.8 (7.3-8.3)
Insulin dose (IU/day)	34 (28-42)	42 (32-58)	39 (32-54)
Insulin dose (IU/kg/day)	0.55 (0.42-0.63)	0.58 (0.52-0.79)	0.56 (0.49-0.72)
IDAA1c (%)	9.4 (9.0-10.4)	10.6 (9.5-11.3)*	10.1 (9.4-10.8)
CV fasting glycemia (%)	46 (40-50)	47 (41-55)	49 (37-54)
Microvascular complications			
Diabetic retinopathy (yes)	25	12	3
Microalbuminuria (yes)	4	4	3
Macrovascular complication [#]	14	4	2
Graft			
Culture time (days)	5 (3-10)	5 (3-10)	4 (2-7)
Number of islet infusions	2	2	2
Beta cell number (10 ⁶ /kg body weight)	2.6 (2.3-3.1)	2.7 (2.0-3.3)	2.6 (2.3-3.4)
Cellular composition (%)			
Beta cells	29 (22-37)	26 (20-35)	24 (24-33)
Alpha cells	8 (5-11)	7 (5-13)	9 (6-14)
Non-granulated cells	49 (41-61)	52 (41-59)	46 (39-49)
Acinar cells	1 (1-6)	2 (1-5)	3 (1-6)
Dead cells	8 (6-11)	9 (7-11)	8 (7-12)

Data are shown as median (IQR).

IDAA1c: Insulin dose-adjusted A1c

CV fasting glycemia defined by 100 X (standard deviation/mean)

[#]Macrovascular complication is defined as a combined endpoint (carotid stenosis, coronary heart disease, and arterial stenosis of the lower limbs

Statistical difference between IS continue group versus graft failure group or stopped due to other reasons group (Mann-Whitney U) * $p < 0.05$, $^{\$}p < 0.005$

Table 2. Number and type of Severe Adverse Events (SAE)

Number of patients with SAE (%)		Number of SAE (%)	Relationship to study	Description	Post-Transplantation	Treatment	Outcome
Total	24 (47)	37 (100)					
Procedure-related	3 (6)	3 (8)	Definitely	-Abdominal bleeding post-laparoscopy -Diaphragm irritation post-percutaneous puncture	Day 1, day 1 Day 5	Transfusion (1)	Resolution
Immune suppression-related	19 (37)	31 (84)	Definitely	- Tacrolimus encephalopathy - Tacrolimus nephropathy	Mo 26 Mo 30	Stop tacrolimus Dose reduction	Resolution
			Probably	CMV infection	Mo 1, 17 Mo 4 Mo 6 Mo 23	IV ganciclovir PO valganciclovir, MMF reduction IV ganciclovir, PO valganciclovir, MMF reduction Stop MMF	Resolution
				Anemia	Mo 0, 1, 4, 15	Transfusion	Resolution
				Opportunistic infection - Candida oesophagitis - Microsporidium	Mo 5 Mo 10	Fluconazole Albendazole and MMF stop	Resolution
				Leucopenia of unknown origin	Mo 9	MMF dose reduction	Resolution
			Possibly	Gastro-intestinal - Gastro-enteritis - Left fossa pain - Chronic diarrhea	Mo 0, 1, 1, 3, 6, 9, 10, 17, 19 Mo 2 Mo 39	Antibiotics (2) Antibiotics and stop MMF	Resolution
				Other viral infections	Mo 23, 25		Resolution
				Diffuse liver metastasis of gastric adenocarcinoma	Mo 27	Chemotherapy	Death
				Acral lentiginous melanoma	Mo 13	Resection, stop immune suppression	Resolution
				Invasive prostate adenocarcinoma	Mo 24	Total prostatectomy	Resolution
				Invasive sigmoid adenocarcinoma	Mo 34	Resection, stop MMF	Resolution
Other	2 (4)	3 (8)	Probably not	Instable angina	Mo 1	CABG	Resolution
				Cerebral hemorrhage	Mo 4	Drainage	Death
				Exercise-induced angina	Mo 18	PTCA	Resolution

No specific therapy is mentioned when symptoms resolved with conservative management

SAE: serious adverse events, Mo: months, MMF: mycophenolate mofetil, CABG: coronary artery bypass graft, PTCA: percutaneous transluminal coronary angioplasty

Table 3. Percent (%) of patients experiencing at least one adverse event under immune suppression therapy during 48 months follow up

Total 48 months follow-up		Month			
		0-12	12-24	24-36	36-48
Number of patients under IS	51	51	38	34	29
Serious Adverse Events	47%	33%	18%	15% *	3% *
Side effects (signs and symptoms)	57%	51%	26% *	21% *	21% *
Depletion of blood cells[#]	100%	100%	66% \$	47% \$	31% \$

[#] Neutropenia and/or lymphocytopenia and/or anemia and/or thrombocytopenia

Statistically significant reduction of adverse events compared with first year PT (PT month 0-12), * $p < 0.05$, \$ $p < 0.001$

Table 4. Prevalence of side effects[€] in non-uremic Type 1 diabetic recipients of long-term cultured islet beta cell allograft under immune suppression

Follow-up PT months	Patients n	Side effects n (%)					
		Gastrointestinal	Neurological	Respiratory	Musculoskeletal	Asthenia	Skin
0-1	51	11 (22)	5 (10)	3 (6)	4 (8)	3 (6)	0(0)
1-12	51	15 (29)	11 (22)	8 (16)	7 (14)	6 (12)	6 (12)
12-24	38	4 (11)*	1 (3)*	2 (5)	4 (11)	3 (8)	3 (8)
24-36	34	3 (9)*	2 (6)	3 (9)	3 (9)	1 (3)	2 (6)
36-48	29	1 (3)*	2 (7)	2 (7)	3 (10)	1 (3)	2 (7)

[€] Grade ≥ 2 symptoms in > 5 percent of patients

PT: post-transplantation

Statistically significant reduction of patients experiencing side effects compared with first year posttransplantation (PT month 0-12), * $p < 0.05$

Table 5. Implant function and metabolic control in diabetic recipients of long-term cultured islet beta cell allograft

			Immune suppression				Metabolic control											
Follow-up	Patient number n		Tacrolimus trough (ng/ml)		MMF dose [§] (mg/day)		C-peptide [#] (ng/ml)		CVfg (%)		HbA1c (%)		Insulin dose (IU/kg/day)		% of patients with Insulin independence		% patients with detectable c-peptide (≥0.2 ng/ml)	
PT months	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Before Tx	29	16					0.09 (0.09-0.09)	0.09 (0.09-0.09)	46 (40-50)	47 (41-55)	7.6 (7.0-8.3)	8.1 (7.5-8.8)	0.55 (0.42-0.63)	0.58 (0.52-0.79)				
0 - 3	29	16	9.5 (8.8-10.2)	10.0 (9.0-10.2)	2000 (2000-2000)	2000 (2000-2000)	0.81 (0.45-1.21)	0.77 (0.48-1.22)	26 (0-36)	26 (20-31)	5.4 (5.0-6.2)	5.7 (5.1-5.9)	0.39 (0.30-0.52)	0.41 (0.22-0.56)	3	6	97	94
3- 6	29	16	8.9 (8.0-10.0)	9.2 (8.0-10.5)	1500 (1000-2000)	1500 (1500-2000)	2.06\$ (1.46-2.50)	1.00 (0.67-1.60)	16* (12-22)	25 (17-34)	6.0 (5.5-6.2)	6.1 (5.6-7.0)	0.16 (0.02-0.29)	0.27 (0.20-0.48)	48	19	100	100
6- 12	29	15	8.7 (8.0-9.1)	7.3 (6.5-10.0)	1500 (1000-1500)	1500 (1500-2000)	1.85\$ (1.07-2.74)	0.53 (0.28-0.97)	16 ^{\$} (11-20)	36 (23-46)	6.4* (5.9-6.8)	6.7 (6.2-7.5)	0.13 ^{\$} (0-0.25)	0.33 (0.20-0.48)	52	13	100	93
12-24	29	8	8.0* (6.9-8.0)	6.5 (4.4-7.6)	1250 (1000-1500)	1500 (1250-1500)	1.39* (0.52-1.93)	0.45 (0.21-1.22)	22 (18-28)	31 (18-42)	6.7 (6.3-7.1)	7.2 (6.4-7.7)	0.20 (0.09-0.30)	0.36 (0.07-0.45)	38	14	100	88
24-36	29	5	7.0 (6.3-8.0)	6.5 (2.2-8.6)	1000 (1000-1500)	1250 (1000-1250)	0.80 (0.52-1.34)	0.19 (0.10-0.95)	26 (23-33)	34 (30-35)	7.0 (6.5-7.5)	7.1 (5.8-7.7)	0.29 (0.17-0.39)	0.43 (0.19-0.53)	14	0	97	40
36- 48	29	0	7.0 (5.0-8.4)		1000 (1000-1500)		0.66 (0.31-1.01)		33 (25-36)		7.2 (6.2-8.0)		0.31 (0.23-0.42)		7		93	

Data are shown as median (IQR)

PT: posttransplantation, IS: immune suppression, MMF: mycophenolate mofetil

A: Under immune suppression for 48 months, B: immune suppression stopped before 48 months due to graft failure

[§] Total 4 patients (3 in IS continue and 1 in IS stop group) were temporarily under mycophenolic acid (Myfortic®) during the first year and switched to mycophenolate mofetil (Cellcept®) thereafter.

[#] Median C-peptide with glycemia between 120-220 mg/dl

Statistically significant compared with Group B * $p < 0.05$, $^{\$}p < 0.005$